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Interlaboratory Comparison Acute Testing Set

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Interlaboratory Comparison

Acute Testing Set

by

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Prepared for Office of Pesticides and Toxic Substances

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A multiple laboratory (6) set of tests was conducted using a single Test Standard Method. The tests consisted of static and dynamic aquatic bioassays with two species of fish and static tests with Daphnia magna in duplicate. The extreme values for any one test were one order of magnitude while the extremes of the means were only a factor of 12.

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a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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Comprehensive Report

Interlaboratory Comparison

Acute Testing Set

Mans additions to the environment, by plan or mistake, of materials not normally found there have increased tremendously. Various offices of the U.S. Environmental Protection Agency are charged with regulation of various sources, i.e., Office of Pesticides Programs. A relatively new unit, the Office of Toxic Substances (OTS), is charged with making judgments on the effect of those materials not regulated elsewhere.

The OTS staff and personnel in the Office of Research and Development (ORD) have been working on a set of toxicity testing protocols to be requested for premarket testing of materials which appear to be potential hazards. Office of Research and Development research labs have performed round-robin tests on such protocols. This report is a compilation of the acute tests which were part of the first group.

Methods and Materials

Protocols for acute static and dynamic (flow-through) tests with fathead minnows and rainbow trout were conducted with a pesticide endosulfan and a metal, silver nitrate ($AgNO_3$). (Each chemical was run in duplicate for each species and test type for a total of 16 tests.) Four contract laboratories and two EPA research laboratories participated. The final data set contains 96 96-hr LC50 values. Also two acute 48-hr tests with Daphnia magna were conducted using the same protocol with slight modifications by each laboratory (12 each 48 hr LC50 values).

The protocol, used was ASTM Draft 7, April 11, 1978, of Proposed Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians.

Contractors were asked to use natural waters and to provide their own fish using the safeguards necessary as per the protocol. Each contract laboratory was visited once by the project officer during actual testing. Data handling in the reports was as requested in the supplied protocol.

To insure as much as possible a normal situation they were asked in the bidding process to supply people with experience in toxicity bioassays, natural waters, and an adequate analytical competence, as per the attached rating sheet (Figure 1). No effort was made to correct small differences in technique or interpretation of the protocol during the duration of the actual testing. Each successful contractor was told to report as per the protocol with a separate section in the report for problems with interpretation. Therefore, each contractor was asked to regard the work and test results as if clarity of the protocol affected the data variance. He would be asked to testify in court as to their validity and to approach all internal decisions on this basis. As an example, if it was found that the analytical work was in question, the contractor was asked to do what was necessary if he was to testify in a court room rather than having the project officer say what he should do.

In this report, 96-hr LC50s used for statistical comparisons, are those reported by the participating laboratories with only random checks as to accuracy.

The evaluation criteria used to select the contract laboratories follows. It was deemed necessary to have experienced people so problems with the protocol would be identified. Therefore a high priority was given to familiarity with aquatic toxicity testing in general and the protocol in particular.

8. Technical Evaluation Criteria:

The criteria to be used in the evaluation of proposals is set

forth below:

Figure 1

		Maximum Points
a.	Qualification of personnel	15
b.	Suitability of water (preference will be given to natural waters)	15
c.	Familiarity with aquatic bioassays	15
d.	Familiarity with ASTM procedures	10
e.	Adequacy of bioassay facilities	10
f.	Availability of analytical equipment	10
g.	Competence of analytical personnel	10
h.	Ability to meet timetable	5

Toxicants

All toxicants were provided by the government. They were reagent grade silver nitrate and technical grade endosulfan. Also provided were purified samples of endosulfan I and II to be used by the experimenters as a analytical standard. The purified reference samples were obtained from the EPA Standards Branch.

Solvents

All of the laboratories dissolved the silver nitrate in distilled water and then added or metered the required amounts into the test containers.

The endosulfan was dissolved in acetone by all but Lab 4. They saturated ultra-pure water by stirring and then filtered and used the filtrate as their highest concentration.

Apparatus and Test Setup

The protocol is not specific concerning the types of apparatus to be used in maintaining temperature in flow through or static tests. The equipment to be used in making up the toxicant water mixtures necessary, is also not specified. Table 1 shows how they were accomplished by each laboratory.

Construction

All laboratories followed the protocol in the use of materials for holding and piping of test waters, test chambers and heating equipment. All test tanks were either glass jars or aquaria constructed with silicone and all piping was rigid PVC or aged black iron. Diluter construction was nearly all glass with a minimum of tygon used for joints.

Chambers

Glass chambers of sufficient size (10 l volume) were used for all static tests.

Glass aquaria made with a minimum of silicone glue and containing 20 to 30 liters volume with covers to prevent fish (trout especially) from escaping were used by all laboratories.

Volume Control

All laboratories maintained suitable volume control in all of the constant flow tests by the use of dilution delivery equipment as noted in Table 1. This is probably the least troublesome of all requirements in the protocol. The usual diluting equipment by its intrinsic nature works best at a rate which delivers 4 to 6 turnovers into a chamber of the required size.

The maintenance of concentration control is considered to be very

Table 1
Laboratory Test Facilities

Lab ID	Temperature Control Method		Water Heating	Flow Through Toxicant Preparation	Holding Tanks
	Static Tests	Flow Through Tests			
1	Temperature control room	Flow rate	Stainless coil HB	Defoe diluter	Flow through polyethylene
2	Water baths	Flow rate	Glass water heater	Mount/Brungs	Flow through glass
3	Trout water bath, Fathead temperature control room	Flow rate and water bath	Glass water heater	Michigan type	Flow through glass
4	Water bath	Flow rate	Glass aquaria heater	Marriot bottle and flow meter	Flow through polyethylene
5	Water bath	Flow rate	Glass water heater	Modified Mount/Brungs	Flow through glass
6	Temperature Control room	Temperature and flow through	Stainless coil HB	Mount/Brungs	Flow through glass

important during bioassay testing. Table 2 has a compilation of the overall concentrations reported by each laboratory comparing their nominal with analyzed concentrations. Most of the variation in initial static and flow through numbers is analytical and appeared in the lower concentrations.

The final static values reflect losses by precipitation, organic binding or in the case of endosulfan, degradation or volatilization.

Table 3 presents a compilation of the source of all test animals of all of the participants. The sources of the trout are most varied with all of them being purchased either as eggs or fingerlings. All except two raised their own fathead minnows and all had lab reared Daphnia.

Water quality parameters for the dilution waters are summarized in Table 7. All participating laboratories except number 2 have soft water. All water samples were from natural sources with no chlorination.

Results

Tables 4, 5, and 6 present the analyzed participating laboratory supplied LC50s for all of the tests (total of 120). These results were used as given by the reporting laboratory.

Random checks were made of the data in those cases where the preferred probit analysis was not used. Results were different only at the third significant digit, (Example 22.5 by probit and 22.3 by Lichfield Wilcoxson) so all results were used as submitted. All data are in micrograms (ug) per liter with 95% confidence interval immediately below the LC50.

Statistical Output

Regressions were run on the test sets vs. various water quality data from the laboratories. No significant correlations were found with the

Table 2: Toxicant delivery and concentration control.[1]
Fish and acute Daphnia

Lab ID	Silver	
	Static Studies Initial	Recovery % Final
1	100% ± 10%	80% ± 25%
2	100% ± 10%	85% ± 30%
3	100% ± 10%	85% ± 20%
4	90% ± 10%	70% ± 10%
5	100% ± 15%	---
6	100% ± 10%	80% ± 20%

	Endosulfan	
	Static Studies Initial	Recovery % Final
1	95% ± 12%	30% ± 70%
2	100% ± 15%	40% ± 100%
3	98% ± 18%	20% ± 30%
4	100% ± 20%	---
5	100% ± 12%	50% ± 50%
6	100% ± 12%	10% ± 50%

	Constant Renewal Studies Mean Recovery %	
	Silver	Endosulfan
1	100% ± 12%	100% ± 10%
2	100% ± 10%	100% ± 10%
3	100% ± 11%	100% ± 15%
4	100% ± 20%	100% ± 12%
5	100% ± 20%	100% ± 15%
6	100% ± 9%	100% ± 12%

[1]Percentages reported were developed by comparing nominal with analytical values as reported by each laboratory.

Table 3

Test Organisms
Source and Sizes Used

	Fathead Minnows		Rainbow Trout		Daphnia	
Lab ID	Size	Source	Size	Source	Age	Source
1	0.5-1.5 g Flow through test	Lab reared	3-10 g Flow through	Genoa WI USFWS hatchery	Newborn	Lab reared
	0.5-0.7 g static tests	Lab reared	1-3 g static	Genoa WI USFWS hatchery		
2	Not given	Ferder Fish hathery Baltic OH	Not given	Beity Resort Valley Wool Spring Creeks Trout hatchery Lewiston MD	Newborn	Lab reared
3	0.5-0.7 g bath	Kurtz Fish hatchery Elvercon PA	1-3 g bath	Wichelfelder trout hatch-ery Boystown PA	Newborn	Lab reared
4	0.05-0.2 g flow through test	Lab reared	1.0-1.5 g flow through test	Mt. Lassen hatchery Red Bluff CA	Newborn	Lab reared
	0.2-0.5 g static test	Lab reared	2.5-3.5 static test	Mt. Lassen hatchery Red Bluff CA		
5	0.5-0.9 g bath	Lab reared	.3-6.5 static	Oregon State hatchery	Newborn	Lab reared
			1-3 g flow through	lab reared		
6	.5-1 g bath	Lab reared	.25-1.0 g bath	Trout Lodge WA	Newborn	lab reared

Table 4

Daphnia Acute Tests 48 hr LC50s ug/l

95% Confidence Interval

LAB	$AgNO_3$ Test 1	$AgNO_3$ Test 2	Endosulfan Test 1	Endosulfan Test 2
1	0.66	0.39*	218	282
	0.46 - 1.3	0.32 - 0.47	91 - 572	245 - 328
2	45	49	250	630
	43 - 47	45 - 54	210 - 300	550 - 720
3	2.2	2.9	740	378
	1.9 - 2.8	2.3 - 3.6	521 - 1051	220 - 645
4	0.90	1.03	266	158
	0.78 - 1.08	0.86 - 1.24	241 - 295	137 - 186
5	1.07	0.64	342	271
	0.86 - 1.55	0.51 - 0.79	263 - 446	225 - 325
6	14.9	8.4	372	328
	Not Recorded			

* Nominal - no chemistry

TABLE 5

Endosulfan 96 hr LC50s for All Fish Tests

Values are µg/l Active Ingredient

Lab ID	FHCF	FHCF	FHS	FHS	RTCF	RTCF	RTS	RTS
1	1.2 .99 - 1.70[b]	1.91 1.19 - 2.96	2.10 1.70 - 5.86	3.45[1] 2.00 - 7.14	.69 .25 - 1.00	.75 .48 - 1.06	1.210[a] .85 - 1.51	.89 .69 - 1.13
2	.29 .19 - .44	.45 .38 - .53	2.10 1.7 - 2.6	3.20 2.7 - 3.8	.17 .12 - 24	.29 .24 - 35	.49 .40 - .60	.80 .64 - 1.0
3	.76 .64 - .90	.73 .60 - .89	1.70 1.42 - 2.04	1.48 1.33 - 1.64	.30 .25 - .36	.27 .17 - .44	1.34 1.11 - 1.62	2.43 2.15 - 2.75
4	.81 .67 - 1.00	.80 .67 - 1.00	1.9 1.82 - 2.03	.97 .78 - 1.19	.26 .23 - 1.00	.41 .33 - 1.00	1.30 1.14 - 1.58	.63 .47 - 1.00
5	1.67 1.54 - 2.34	1.57 0 - 2.57	1.35 .75 - 2.44	1.20 .82 - 2.19	.32 .27 - .38	.42 .36 - .49	1.69 .77 - 3.69	1.63 1.37 - 1.93
6	.75 .54 - .84	1.00 .8 - 1.1	3.20 2.9 - 3.7	2.50 2.2 - 3.0	.26 .23 - .29	.24 .19 - .34	.69 .65 - .75	.79 .72 - .89

[a] Nominal no analysis

[b] All secondary values are 95% confidence intervals.

FHCF = Fathead minnow constant flow test

FHS = Fathead minnow static test

RTCF = Rainbow trout constant flow test

RTS = Rainbow trout static test

TABLE 6
Silver 96 hr LC50 for all Fish Tests
Values are ug/l Ag^{++}

Lab ID	FHCF	FHCF	FHS	FHS	RTCF	RTCF	RTS	RTS
1	10.98 9.31 - 12.77[d]	11.75 10.01 - 11.99	30.43 24.07 - 35.88	22.66 18.81 - 27.09	17.87 12.80 - 28.75	16.38[a] 12.80 - 19.20	19.92 16.10 - 31.53	31.80 26.1 - 36.2
2	150 130 - 180	110 100 - 130	230 180 - 290	270 220 - 320	240 200 - 290	170 150 - 200	240 180 - 320	280 220 - 360
3	11.1 8.6 - 14.3	8[b]	13.8 11.4 - 16.7	19.6 17.7 - 23.8	14 12.2 - 16.1	12[c]	48 42.9 - 53.8	54 36.5 - 80.0
4	5.3 4.92 - 5.73	3.9 3.52 - 4.22	6.7 5.20 - 8.62	12.3 11.40 - 13.50	6.9 6.48 - 7.40	8.4 5.94 - 11.91	11.8 9.9 - 14.0	108.9* 8.39 - 180.2
5	5.78 0 - 7.19	5.6 4.52 - 6.38	12.4 7.45 - 12.4	9.74 8.49 - 11.19	8.5 7.10 - 10.2	9.72 8.36 - 11.34	72.89* 60.3 - 88.3	84.4* 70.9 - 100.2
6	6.3 5.8 - 6.8	5.0 4.2 - 5.7	10.3 9.6 - 11.1	8.7 7.8 - 9.6	11.5 10.8 - 11.8	9.7 9.0 - 10.3	24.6 23.6 - 25.8	22.5 20.9 - 24.7

[a] Nominal

[b] Estimate less than 50% killed at highest concentration.

[c] Estimate more than 50% dead at lowest concentration.

[d] All secondary values are 95% confidence intervals

* Heavy aeration.

FHCF = Fathead minnnow constant flow test

FHS = Fathead minnow static test

RTCF = Rainbow trout constant flow test

RTS = Rainbow trout static test

Table 7
Laboratory Water Quality Parameters[a]

Parameter	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
Aluminum	0.05	<0.01		0.05		
Arsenic		<0.001	0.0116	<0.01		
Barium	0.015			<0.25		
Boron				0.07		
Cadmium	<0.0001	<0.001		<0.002	<0.005	
Calcium	13.5	24.5		11.6	14.7	31
Chromium	0.01	0.001		<0.005	<0.005	
Copper	0.002	<0.01	0.0025	0.01	<0.005	
Iron	0.05	0.012	<0.01	0.09	0.01	
Lead	0.015	0.009	<0.005	<0.02	<0.04	
Magnesium	3.2			4.1	3.9	4.2
Manganese	0.08			<0.005	<0.005	
Mercury	<0.00001	<0.0001		<0.0005	<1.0	
Potassium	0.52			0.8	0.50	2.1
Selenium				<0.0025		
Silver				<0.005		
Sodium	1.1			6.3	6.1	8.9
Zinc	0.002	<0.01	0.009	0.01	<0.005	
Bicarbonate				45.5		
Carbonate				0.0		
Chloride	1.2	32	8.0	11.1	13.0	1
Fluoride				0.09		
Hydroxide				0.0		
Nitrate		0.15		0.2	NO_2 +NO_3 0.49	
Nickel			0.026			
Phosphate		0.10		<0.01		
Sulfate				4	13	3
ABS				<0.05		
NH_3		<0.05	0.08	<0.05	<0.005	
Dissolved O_2		9.3		8.7		
SiO_2				6.0		
Hardness	48	255	54	46.1		75
Alkalinity	45		1.4	37.3		90
Conductivities (mhos/cm)			72	136		190
pH	7.74	7.8	6.8 - 7.1	7.6		6.7-8.8
Turbidity				1.5	3	
Color				0		
Chlorinated Hydrocarbons		<0.008		<0.0001		
Organophosphorus Pesticide		<0.002		<0.002		
Source	Lake Superior	Deep Well	Spring	Reservoir	Well	Well

Units in mg/l except for those not appropriate (pH, conductivity, turbidity, color)

< equals level of detection

[a]Values are as reported by Round Robin participant laboratories

Table 8

Part A
Interlaboratory
Analysis of Variance for Silver Results
Fish Only

Due to	DF	SS	MS=SS/DF
Laboratories	5	251407.	50281.
Test Types	3	14294.	4765.
Combined	15	17328.	1155.
Error	24	5086.	212.
Total	47	288115.	

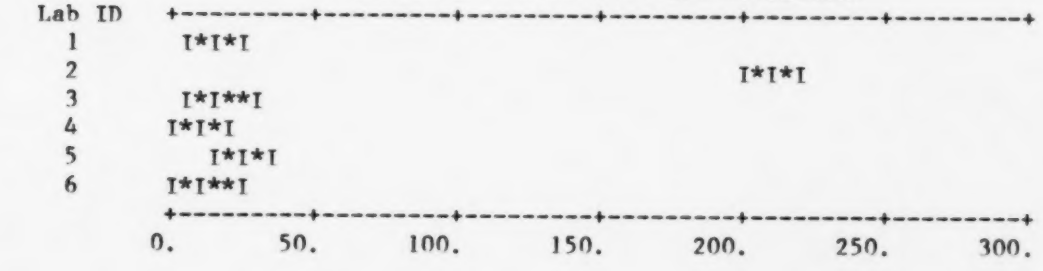
Part B
Cell Means

Rows are Levels of Laboratories Columns are Levels of Tests

Lab ID	FHCF	FHS	RTCF	RTS	Row Means
1	11.4	26.5	17.1	25.9	20.2
2	130.0	250.0	205.0	260.0	211.3
3	9.6	14.9	13.0	51.0	22.1
4	4.6	9.5	7.7	11.3	8.3
5	3.2	11.1	9.1	78.6	25.5
6	5.6	9.5	10.6	23.6	12.3
Col. Means	27.4	53.6	43.7	75.1	49.9

Pooled St. Dev. = 14.6

Part C
Individual 95 Percent C. I. for Level Means of Laboratories
(Based on Pooled Standard Deviation)



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Individual 95 Percent C. I. for Level Means of Tests
(Based on Pooled Standard Deviation)

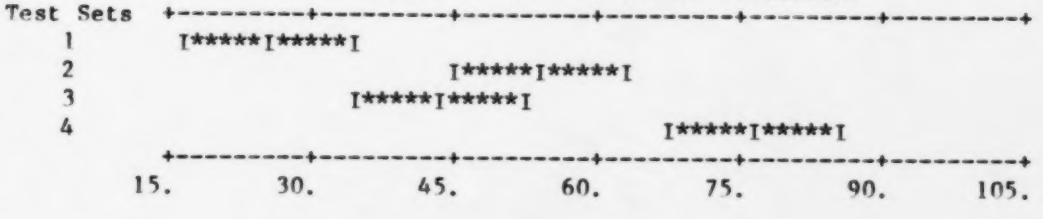


Table 9

Part A
Interlaboratory
Analysis of Variance for Endosulfan
Results of Fish Only

Due to	DF	SS	MS=SS/DF
Laboratories	5	1.999	.400
Test Types	3	19.418	5.139
Combined	15	8.688	.579
Error	24	3.481	.145
Total	47	32.586	

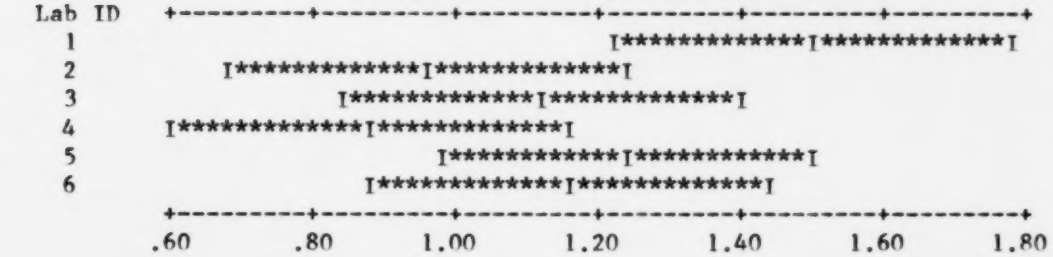
Part B
Cell Means

Rows are Levels of Laboratories Columns are Levels of Tests

Lab ID	FHCF	FHS	RTCF	RTS	Row Means
1	1.555	2.775	.720	1.050	1.525
2	.370	2.650	.230	.645	.974
3	.745	1.590	.285	1.885	1.126
4	.805	1.435	.335	.965	.885
5	1.620	1.275	.370	1.660	1.231
6	.875	2.850	.250	.740	1.179
Col. Means	.995	2.096	.365	1.158	1.153

Pooled St. Dev. = .381

Part C
Individual 95 Percent C. I. for Level Means of Laboratories
(Based on Pooled Standard Deviation)



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(Based on Pooled Standard Deviation)

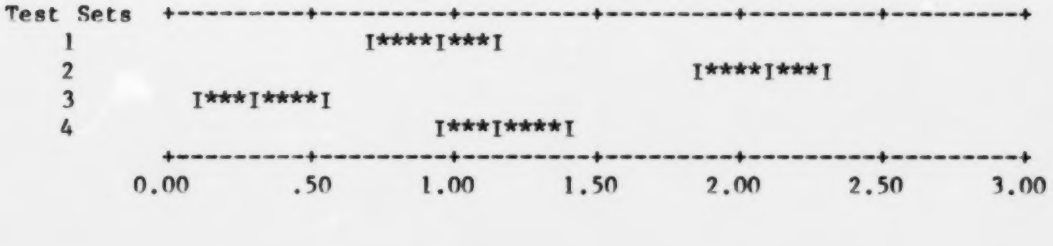


Table 10

Part A
Analysis of Variance for Silver
Results at all Acute Tests

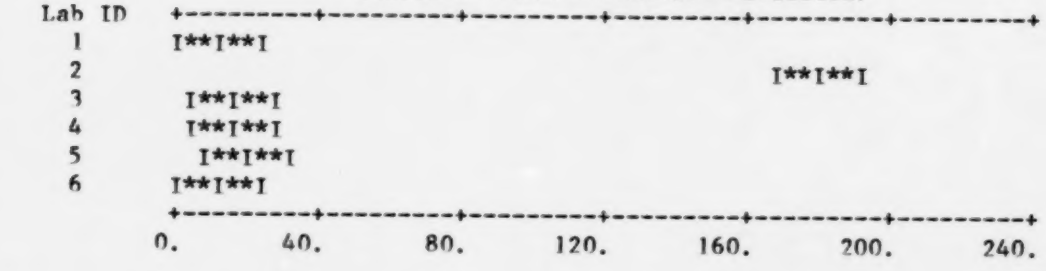
Due to	DF	SS	MS=SS/DF
Laboratories	5	217661.	43532.
Test Type	4	36184.	9046.
Combined	20	45444.	2272.
Error	30	9832.	328.
Total	59	309120.	

Part B
Cell Means
Rows are Levels of Laboratories Columns are Levels of Tests

Lab ID	FHCF	FHS	RTCF	RTS	Daphnia	Row Means
1	11.4	26.5	17.1	25.9	.5	16.3
2	130.0	250.0	205.0	260.0	47.0	178.4
3	9.5	16.7	13.0	51.0	2.5	18.6
4	4.6	9.5	7.6	60.3	1.0	16.6
5	5.7	11.1	9.1	78.6	.9	21.1
6	5.6	9.5	10.6	23.6	11.7	12.2
Col. Means	27.8	53.9	43.7	83.2	10.6	43.9

Pooled St. Dev. = 18.1

Part C
Individual 95 Percent C. I. for Level Means of Laboratories
(Based on Pooled Standard Deviation)



Part D
Individual 95 Percent C. I. for Level Means of Tests
(Based on Pooled Standard Deviation)

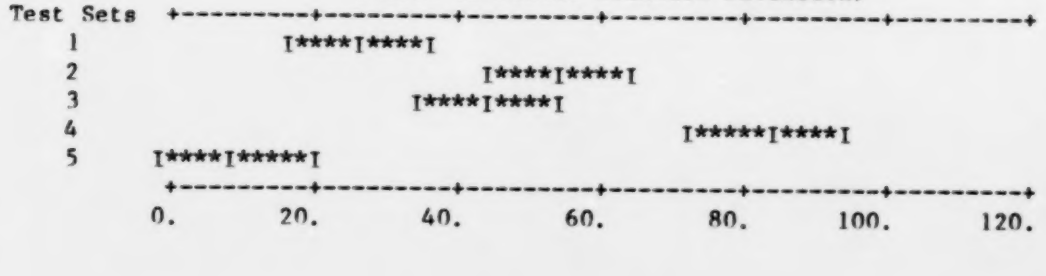


Table 11

Part A
Analysis of Variance for Endosulfan
Results at all Acute Tests

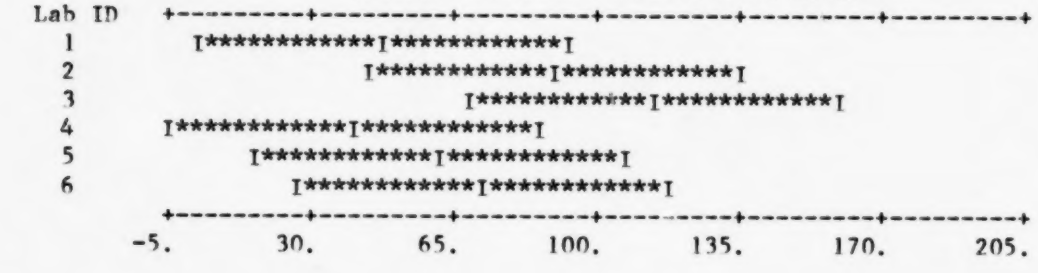
Due to	DF	SS	MS=SS/DF
Laboratories	5	32987.	6597.
Test Type	4	1187898.	296974.
Combined	20	132356.	6618.
Error	30	149094.	4970.
Total	59	1502335.	

Part B
Cell Means
Rows are Levels of Laboratories Columns are Levels of Test Types

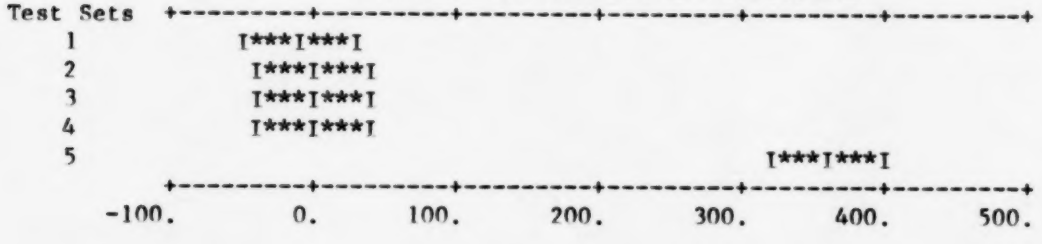
Lab ID	FHCF	FHS	RTCF	RTS	Daphnia	Row Means
1	1.6	2.8	.7	1.0	250.0	51.2
2	.4	2.6	.2	.6	440.0	88.8
3	.7	1.6	.3	1.9	559.0	112.7
4	.8	1.4	.3	1.0	212.0	43.1
5	1.6	1.3	.4	1.7	306.5	62.3
6	.9	2.8	.2	.7	350.0	70.9
Col. Means	1.0	2.1	.4	1.2	352.9	71.5

Pooled St. Dev. = 70.5

Part C
Individual 95 Percent C. I. for Level Means of Laboratories
(Based on Pooled Standard Deviation)



Part D
Individual 95 Percent C. I. for Level Means of Tests
(Based on Pooled Standard Deviation)



various common water quality parameters and endosulfan toxicity. The silver toxicity had a 95% or greater correlation with hardness and alkalinity with all tests except the rainbow trout statics which were about 80% indicating other factors involved. Only slight correlation was noted with pH.

A two way Analysis of Variance (AOV) was computed with test type means vs. laboratories being considered. AOV outputs for silver and endosulfan data are found in Table 8 and 9respectively for fish acutes only and in Table 10 and Table 11 for all fish and Daphnia acute tests in combination.

Discussion

General

Literature search efforts of the staff of the Environmental Research Laboratory-Duluth as part of the criteria document process uncovered widely scattered LC50 values for some materials. (Brungs, Personal communication). Expectations for this bioassay interlaboratory comparison were in the range of one order of magnitude considering the freedom of investigators for source of test animals, water quality, investigators experience and other less tangible factors.

Statistical

The analysis presented in Tables 8-11 show values with much less variation than expected. The data used are the reported LC50s from Table 5 and 6 respectively. The number presented in each column in Part B of each table is the mean of the two tests of a like condition or species for the lab in the left column i.e. 1-1 is the mean of the two Fathead Constant Flow tests for laboratory 1. The Column Means are the means of all like tests across all laboratories and the Row Means are the means of all tests from each laboratory. Part C of Tables 8-11 shows graphically the laboratory means of all tests $\pm$ the pooled standard deviation and enables visual comparison of data output between laboratories.

Visual comparisons of tests types are presented in Part D of Tables 8-11. Scales of Part C and Part D of Tables 8-11 are different. The graphs (Part C) show that all of the laboratories overlap ranges except lab 1 and lab 3 for endosulfan. The means of these two laboratories at 1.48 µg/l and 0.89 µg/l are less than a factor of two for all tests. This compares with the means of all constant flow rainbow trout tests of 0.39 µg/l versus 2.117 µg/l for the fathead statics for a difference of just over 5 times.

Interlaboratory vs. Species

Within species comparison of Static and Constant Flow tests are similar, being 2.3 times (0.92 vs. 2.12) for fathead minnows and 2.97 times (0.385 vs. 1.162) for the rainbow trout. These values are based on the initial analyzed concentration of the static tests and on the analyzed mean of the initial and final concentrations of flow-through tests. Analysis of the end of the static tests reveal a 30%-80% loss of toxicant making an even wider difference in test types if the means were used. Therefore, even by the most conservative estimates there is more difference between tests, than within a test between laboratories, when water quality does not affect the results. Silver values which are affected by water quality, especially hardness, (see Figure 1) has the between test values of slightly less than 3 with the within species tests differences both being 2. However the effect of the water quality (255 mg as $CaCO_3$ vs 30-80 mg as $CaCO_3$) far exceeds this being 211 µg/l Ag total vs 12 to 25 mg for the soft waters. This is approximately a 150 fold difference.

The addition of the Daphnia acute data for silver (Table 11 Part C) makes the ratio between hard and soft waters approximately the same, but the between test ratios become larger (Part D), increasing to a maximum of just under seven (11 vs. 76). In the case of the endosulfan the species

difference is so great (approximately 200 times) that the effect is similar to that of hardness with silver. This species difference compresses the difference between the fish tests, as the hardness does between the laboratories tests, where waters are approximately the same.

Variability Considerations

Potential sources of laboratory test variability include; fish source, fish size, water quality, test chamber aeration rate, method of toxicant preparation and method of data analysis. Water quality, discussed previously, causes wide variations if the toxicant under test is labile to water hardness differences i.e. silver or other heavy metals. Fish size is a factor especially in static testing. Several of the laboratories were found to use larger than recommended fish when those obtained early in the test program grew and replacements were not available. These laboratories No. 3 and No. 5 report some of the highest values for silver. The larger fish required heavy aeration to hold the oxygen at the recommended level and the two factors interacting appear to account for the decreased toxicity. Laboratory 5 accomplished another test with smaller fish and no aeration after the deadline for the regular test set and reported an LC50 of 12 ug/Ag^{++}/l in comparison to the mean of 78 ug/Ag^{++}/l reported earlier. The varying sources of test fish appear to contribute little bias to the results.

As noted, laboratory 2 prepared its endosulfan toxicant solutions by making a saturated water solution and then diluting this to make the concentration series. Determining whether this had any real effect is difficult. Although they report the lowest mean for all of the endosulfan tests, in both of the fathead static tests they are near or equal to the least toxic values.

Much of the cost of conducting bioassays is in the support required for analysis of test conditions. Table 12 presents the comparison of LC50s using

the analyzed data of all concentrations vs. the nominal numbers of each concentration for laboratory 1. Complete probit and binominial analyses were accomplished separately rather than making a conversion of the analyzed answer. Comparison of all sets of data (16 sets) where the nominal and analyzed values are available shows an extreme value of 47% where the analyzed value is higher and 30% where the analyzed value is lower. These are far below the 10 to 200 times shown between species. Although some analytical work is necessary to verify the concentration at the LC50 level, the point of diminishing returns is reached very early when the utility of third decimal place accuracy is measured against the species difference. With acute tests environmental protection is not aided significantly by increased analytical precision if testing of diverse species suffers due to budget constraints.

Another potential source of variability is the statistical workup of the data. All the laboratories except laboratory 4 used the probit analysis as a similar machine method. Laboratory 4 used the Lichfield Wilcoxson hand graph estimate method. As a check on this and to verify the various methods all of their data was recalculated from the raw survival numbers using the supplied analyzed concentrations. In all but 2 cases, Fathead Constant Flow 2 and Rainbow Trout Constant Flow 2, data was obtained by at least one other method. The largest difference found was 16 vs. 19.6 for the Fathead Static 2. The largest error is 22%. All others were less than 10%.

Several results need further clarification. The apparent high results 108.9 ug/l for laboratory 4 in the second rainbow trout static is a case in point. The participating laboratory noted this result. They checked all of their analysis and preparation of toxic solutions and found no apparent errors. No laboratory was either low or high in results with both chemicals

indicating that randomization of analytical and bioassay procedures was taking place as expected. Upon receipt of this report this author asked them to have another person on their staff run another check with the same results. Further perusal of the report of laboratory 4 indicates that perhaps the first result is slightly low and the second slightly high as the mean is very close to that of other participating laboratories. Another data set is that of Lab 2 in the silver data. As mentioned briefly previously this laboratory used hard well water of about 300 mg/l as $CaCO_3$ while all of the rest of the participating organizations had water of 100 mg/l $CaCO_3$ or less.

Metal toxicity is affected by water quality as shown by various reports. A report edited by Andrew et. al. (1975) presents an excellent discussion as to the reason for this, and the data from laboratory 2 is in agreement.

Special Considerations

1. If metals are to be tested, use of the results must be equated with the type of water in question whether as test or receiving water.
2. Animal size is quite critical as indicated in the protocol, but following this part of the method is much easier with a species that is small naturally than with one which rapidly becomes larger i.e. fathead minnows vs. rainbow trout.
3. Constant flow tests produce results with less variability because they are not subject to fluctuating conditions of various factors, such as aeration, precipitation and volatilization.

4. Trout have a propensity for jumping out of test containers necessitating covering of the containers, making observations and sampling more difficult.

5. Every effort should be made to test as many species as possible if protection of the environmental life forms is to be accomplished The large difference between species of over 200 times is much greater than either the variation in test type results or between laboratory results of two to five times.

References

Andrew, R. W., P. V. Hodson, and D. E. Konasewich (Editors). Toxicity to Biota of Metal Forms in Natural Waters. Proceedings of a Workshop. Duluth, MN October 7-8, 1975. Published by Standing Committee on the Scientific Basis for Water Quality Criteria of the International Joint Commission.

Table 12
Nominal vs. Measured Concentrations
ug/l Silver
Laboratory 1

Nominal	Measured	Nominal	Measured
	Silver		
FHCF 1		FHCF 2	
11.28 10.09 - 12.06	10.98 9.31 - 12.77	12.22 11.02 - 13.55	11.75 10.01 - 11.99
FHS 1		FHS 2	
33.50 28.12 - 40.13	30.43 24.07 - 35.88	24.15 20.15 - 28.90	22.66 18.81 - 27.09
RTCF 1		RTCF 2	
23.50 21.3 - 26.1	17.87 12.80 - 28.75	16.38 12.80 - 19.20	no analysis
RTS 1		RTS 2	
28.74 20.80 - 38.80	19.92 16.10 - 31.53	32.95 27.7 - 36.9	31.80 26.1 - 36.2
Daphnia 1		Daphnia 2	
.39 .32 - .47	no analysis	.63 .45 - 1.5	.66 .46 - 1.30
	Endosulfan		
FHCF 1		FHCF 2	
1.24 1.0 - 1.6	1.20 0.99 - 1.70	1.01 0.88 - 1.19	1.91 1.19 - 2.96
FHS 1		FHS 2	
2.35 1.93 - 5.08	2.10 1.70 - 5.86	3.34 2.35 - 4.17	no analysis
RTCF 1		RTCF 2	
0.86 0.62 - 1.0	0.69 0.25 - 1.08	0.81 0.62 - 1.0	0.75 0.48 - 1.06
RTS 1		RTS 2	
1.21 0.85 - 1.51	no analysis	0.94 0.73 - 1.17	0.89 0.69 - 1.13
Daphnia 1		Daphnia 2	
220 188 - 257	218 91 - 527	251 215 - 297	282 245 - 328

Table 13
Comparison of Calculations by Various Methods
Using the Same Data Set
Laboratory 4

Test	Lichfield Wilcoxson	Binomial	Moving Average Angle	Probit
		Silver		
FHCF 1	11.1	<19.3	10.7	10.8
FHCF 2	8est	not suitable	not suitable	not suitable
FHS 1	13.8	18	13.3	13.4
FHS 2	16.0	18.9	19.4	19.6
RTCF 1	14	13.1	13.5	13.6
RTCF 2	12est	<15.2	unsuitable data	unsuitable data
RTS 1	48	unsuitable data	46.6	47.6
RTS 2	54	unsuitable data	unsuitable data	54.5
		Endosulfan		
FHCF 1	0.76	0.64	0.68	0.71
FHCF 2	0.73	0.72	0.72	0.73
FHS 1	1.7	below 2.49	1.71	1.63
FHS 2	1.48	1.52	1.47	1.48
RTCF 1	0.30	below 56	0.29	0.29
RTCF 2	0.27	below 0.62	not suitable	0.23
RTS 1	1.34	1.18	1.23	1.27
RTS 2	2.43	above 1.72	2.37	2.37

Conclusions

1. Laboratory studies accomplished by knowledgeable personnel following a standard protocol with reasonable precision, should produce results which are within the standard deviation of similar tests of like nature accomplished by a single laboratory.

2. Quality of the test waters should be of concern when the test material is known to have a chelating effect as that found with metals and hard water or with the effect of pH as with some organics such as organophosphate insecticides. If nothing is known of these type of reactions of the test material multiple tests with different waters would be expedient.

3. Careful attention to detail as for example fish size has more effect on reproducibility than any protocol by itself providing such protocol is biologically complete.

4. Biolocial effectiveness and completeness should have top priority as poor biological information cannot be improved by statistical analysis but good biological work cannot be harmed by poor statistical work providing the raw unworked data is also presented.

5. If limited funds are available the most information for protection of the environment can be gained by doing short term tests on more and diverse species rather than by expenditure of funds to get a more precise answer for a single species. A longer term test is not

warrented unless the species in question is of particular interest in a certain situation. Long term tests with Daphnia magna with endosulfan (reported elsewhere by Nebeker) would not have predicted the values for the fish.